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The effect of reduced *Artemia* and rotifer use facilitated by a new microdiet in the rearing of barramundi *Lates calcarifer* (BLOCH) larvae

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Abstract

Since live feeds are costly and variable in nutrition, early weaning onto artificial microdiet is advantageous. Thus, we tested a new commercial microdiet using several rearing protocols aimed at reducing live feed inputs. Barramundi *Lates calcarifer* (BLOCH) larvae were reared from 2 days post hatch (dph) to 28 dph and their survival, growth and stress tolerance were examined. Six protocols with 4 replicates were set combining different protocols that progressively excluded live feeds (rotifers and *Artemia*). Rearing protocols using no rotifers (protocol-G) and feeding rotifers for 3 (G3), 7 (G7) and 12 (G12) days were conducted with a 3 day weaning period onto Gemma Micro (Skretting) microdiet followed by feeding microdiet solely. According to a common rearing protocol, 2 protocols received rotifers for 12 days, *Artemia* for 9 days and either Proton (INVE) as control (P12A) or Gemma Micro (G12A) co-fed from 6 dph. Protocols G12 and G12A were reared until 36 dph in order to compare growth post weaning.

Barramundi larvae development was affected by rearing protocols, with co-feeding rotifers and Gemma Micro allowing complete replacement of *Artemia*. Significantly better growth and similar larvae survival and health was achieved compared to co-feeding rotifers, *Artemia* and Proton. By including *Artemia* in the protocol with Gemma Micro survival was significantly improved, but growth was inhibited to 28 dph. Due to compensatory growth in protocols G12 and G12A, whereby accelerated growth occurs in response to previous poor nutrition caused by the weaning process, larvae grew equally well to 36 dph in these protocols. Larval growth and survival positively correlated with the number of days that rotifers were fed in those protocols that did not receive *Artemia*. High lipid levels and relatively higher amino acid leaching rates were considered contributing factors to achieving better larvae growth and survival.

We found that the best protocol for rearing barramundi larvae involves feeding enriched rotifers from initial exogenous feeding to 3 days after the initiation of stomach differentiation at 5 mm standard length (SL), and including supplemental levels of enriched *Artemia* from 5 mm, to post metamorphosis at 12 mm. Co-feeding barramundi larvae on microdiet (i.e. Gemma Micro) should start when the larvae reach 5 mm, in order to establish microdiet early and allow a more efficient weaning process.
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Keywords: Microdiet; *Artemia*; Rotifer; Co-feeding; Weaning; Larvae and barramundi *Lates calcarifer* (BLOCH)

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1. Introduction

Larval nutrition is a key element for the successful culture of marine fish and live feed such as rotifers (*Brachionus plicatilis*) and brine shrimp (*Artemia* spp.) are considered obligatory for success (Baskerville-Bridges and Kling, 2000; Sorgeloos et al., 2001). Live feed organisms are thought to stimulate larval feeding by their movement and metabolic wastes and chemical attractants (mainly free amino acids) (Hart and Purser, 1996; Kolkovski et al., 1997b, 2004; Cahu and Zambonino Infante, 2001; Kolkovski, 2001). Moreover, live feed organisms are more readily digested than microdiets and contribute to the digestive process by providing exogenous enzymes (Baskerville-Bridges and Kling, 2000). However, the high infrastructure and labor requirements along with the variable costs and nutritional value of live feeds (Jones et al., 1993; Reitan et al., 1997; Cahu and Zambonino Infante, 2001; Callan et al., 2003; Langdon, 2003), illustrates the need to develop better adapted replacement microdiets that can supply a reliable and constant source of bioavailable nutrients to the undeveloped larvae (Baskerville-Bridges and Kling, 2000).

In the past, early weaning has led to poor growth and inferior quality larvae with an increased risk of skeletal deformities (Person-Le Ruyet et al., 1993; Cahu and Zambonino Infante, 2001). Recent advances in microdiet formulation have considerably reduced the pre-weaning period allowing the introduction of specific larval diets to marine finfish culture as early as mouth opening (Cahu and Zambonino Infante, 2001). 'Co-feeding' weaning protocols, simultaneously using inert and live diets, allow an earlier and more efficient change over period onto microdiet from live feeds (Hart and Purser, 1996; Daniels and Hodson, 1999; Koven et al., 2001). This method provides higher growth and survival than feeding either live feeds or microdiets alone (Kolkovski et al., 1995). Early co-feeding of an appropriate microdiet will improve larval nutrition and can condition the larvae to accept microdiet more readily, thus preventing an adverse effect on subsequent growth following weaning (Rosenlund et al., 1997; Canavate and Fernandez-Diaz, 1999; Cahu and Zambonino Infante, 2001; Kolkovski, 2001).

In an effort to make the first step in eradicating the need for live feed, i.e. *Artemia* replacement; several commercial diets have been developed. To assess the potential of a new commercial microdiet and show prospective for reduced costs and simplification of larval production, the limits of live feed elimination from a common rearing protocol used in barramundi hatcheries in Australia have been tested. We investigated the effect of the rotifer-feeding period and the exclusion of *Artemia* on barramundi *Lates calcarifer* (BLOCH) larvae growth and survival, by using co-feeding principles with two commercially available microdiets.

2. Materials and methods

2.1. Experimental design

Commercial microdiets with different particle sizes, Gemma Micro 150 and 300 (120–200 and 200–450 μm, respectively), along with Gemma 0.3 (300–600 μm) (Skretting) and Proton 1, 2/3, 2/4 (100–200, 200–400 and 300–500 μm, respectively) (INVE) were used in the experiment (Fig. 1).

Six protocols with 4 replicates were set combining different protocols that progressively excluded live feeds (rotifers and *Artemia*). Rearing protocols using no rotifers (protocol-G) and feeding rotifers for 3 (G3), 7 (G7) and 12 (G12) days were conducted with a 3-day weaning period by Gemma Micro followed by feeding microdiet solely. According to a common rearing protocol (Bosmans et al., 2005), 2 protocols received rotifers for 12 days, *Artemia* for 9 days and either Proton as control (P12A) or Gemma Micro (G12A) co-fed from 6 dph (Fig. 1).

2.2. Larvae

Barramundi larvae were transported at 1 dph from The Darwin Aquaculture Centre, Darwin, Northern Territory, to the Aquaculture Development Unit, Challenger TAFE, Fremantle, Western Australia. After arrival the larvae were kept in an aerated 1000 l holding tank from which they were evenly distributed to the experimental tanks using volumetric allocation.

A total of 195 000 larvae (2 dph) were transferred into twenty-four 270 l up-welling conical tanks (8100 fish $tank^{-1}$) in the system described by Kolkovski et al. (2004) and were fed according to the rearing protocols (Fig. 1). Initially 100 larvae in total and thereafter every 4 days, 5 larvae from each tank were randomly removed and sacrificed using iced water. These larvae were measured for standard length (SL) and wet weight (WW). WW was measured using a four decimal place balance (AND, Japan) after rinsing larvae with de-ionized water on a 400 μm screen and blotting excess moisture away from behind the screen using lint free paper towel. At the final observations (28 dph) 20 fish were sampled from each tank. The developmental stage of larvae was defined following Walford and Lam (1993).

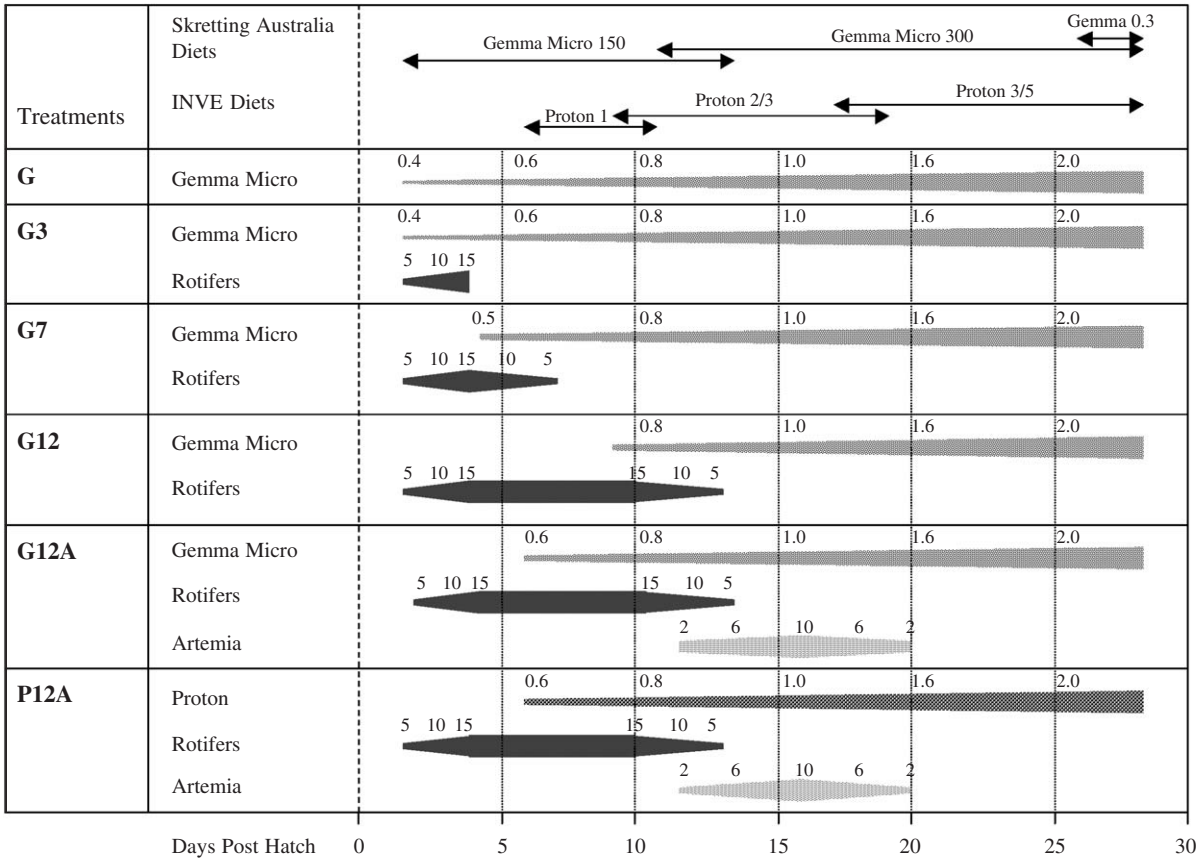

Fig. 1. Rearing protocols of barramundi larvae from 2 to 28 dph. Microdiet feeding rations are expressed as 'g (1000 larvae)$^{-1}$ day^{-1}', rotifers are expressed as 'number of rotifers ml^{-1}' continuously and *Artemia* are expressed as 'number of *Artemia* ml^{-1} day^{-1}'. Temporal microdiet size class distribution is shown for Skretting and INVE diets, with a 3-day overlap for each transitional period. Microdiet feeding regimes are common for all protocols, but starting at different times.

2.3. Rearing protocol

Algae at 300 ppm (concentrated *Chlorella vulgaris*, DHA-enriched SV12®, *Chlorella* Industry Co. Ltd., Japan) and rotifers (cultured with *C. vulgaris* at 500 ppm for 22 h) were automatically supplied by the feeding system described by Kolkovski et al. (2004) to the protocols receiving rotifers (Fig. 1). Protocols not receiving rotifers were automatically administered *C. vulgaris* diluted to 300 ppm until 13 dph using an identical parallel system, after which all protocols stopped receiving algae. Enriched (DC DHA Easy Selco®) *Artemia* spp. (GSL, INVE) were manually supplied 4 times daily to protocols P12A and G12A, and microdiet was administered automatically every 6–12 min (increasing frequency with increased daily ration) using the automatic feeders described by Kolkovski et al. (2005). At 9 am and 4 pm the feeders were checked and a manual feed was given to the tanks from the daily ration (Fig. 1). The system supplied temperature controlled marine bore water at a flow rate of 1.0 l min^{-1} and pumped algae (and rotifers when required) every half-hour at 0.7 l min^{-1}. The physiochemical parameters (average±SE) were maintained at water temperature 28.7±0.8 °C, pH 8.00±0.02, DO 6.5±0.5 ppm, salinity 35.0‰ and a photoperiod of 12 h light/12 h dark. The green water method (Moretti et al., 1999) was used and a secchi disk depth of 80±17 cm was maintained.

2.4. Stress test

Following the trial (at 28 dph), stress tests using exposure to air were conducted (Koshio et al., 1997; Sakakura et al., 1998). The tests were carried out by pouring 200 ml of water containing 10–15 larvae from each tank using a plastic beaker through a mesh (400 μm) screen in order to capture the larvae on the screen. The screen was then blotted dry from below with paper towel. The larvae were left on the dried screen for 9 min and then returned to 8 l of aerated water in a

plastic bucket. The proportion of survivors after 15 min was recorded. The time of air exposure was determined using preliminary observations on the control group for times 6–12 min; approximately half of the control group fish died after 9 min and therefore this time period was used in the test. The average survival for each protocol was calculated by averaging the outcomes from 4 replicates.

2.5. Grow-on period

Larvae from protocols G12 and G12A were graded at 28 dph through a stainless steel grill grader set to a 2.5 mm gap in order to remove very fast growing fish, to avoid cannibalism. 362 of the remaining larvae were restocked into each of 6 tanks. The flow rate was increased to 3 l min^{-1} and the tank flow direction was reversed to bottom draining. A 1 mm screened standpipe was installed along with a protein skimmer at the water surface (Kolkovski et al., 2004). The fish were fed Salmon Starter Crumble (0.6 mm, Skretting) diet at 10% fish WW per day, increasing at a daily increment of 10% of the previous days' ration. At 36 dph, 10 fish from each tank were measured for SL and WW.

2.6. Growth rate indicator

Daily Growth Coefficient (DGC) (Kaushik, 1998) was calculated using the following equation:

$$\mathrm{DGC} = \frac{[(\text{Final weight})^{0.33} - (\text{Initial weight})^{0.33}]}{\text{Number of Days}}$$

2.7. Diet leaching

Soluble amino acid levels were determined by adding approximately 1 g (weighed to 4 decimal places) of each diet into a beaker containing 500 ml of marine bore water at 21 °C. The samples were stirred using an automatic overhead stirrer (IKA Labortechnik, RW20) with a 25 mm propeller type agitator set to 800 rpm. The stirrer was set to keep the majority of microdiet particles suspended in the water column for the duration of the leaching process (Yúfera et al., 2002). The microdiets were mixed for 8 min less filtering time, so that the moisture was removed from the diets at 8 min ± 5 s. Deionized water was used to wash the sample at the end of the filtering process. Paper filters with 11 μm pore size were used to filter the microdiets. The supernatant was then reserved and analyzed to determine the concentration of free amino acids.

Amino acids were separated on a reverse phase amino acid HPLC column using an 1100 series HPLC system (Agilent, USA) and measured by precolumn derivatization using *o*-phthalaldehyde (OPA) and 9-fluorenylmethylcloroformate (FMOC) (Sigma-Aldrich). These results were then used to calculate the amount of free amino acids (FAA) per gram of microdiet, using the following equation:

$$\text{FAA (nmol g}^{-1}) = \frac{\text{FAA conc. (umol ml}^{-1}) \times \text{supernatant volume (ml)}}{\text{microdiet dry weight (g)}}$$

2.8. Proximate analysis

Total nitrogen was determined with a Technicon segmented flow analyser using the Berthelot colorimetric determination (Searle, 1984), after a sulphuric/salicylic acid/hydrogen peroxide digestion of the organic material (Bradstreet, 1965). Crude protein was determined by multiplying the nitrogen content by a conversion factor of 6.25 (Sosulski and Imafidon, 1990).

A Soxhlet *n*-hexane extraction (method 963.15, AOAC, 2000) was used to determine crude fat.

2.9. Statistical analyses

Statistical analyses of protocol effects were performed using SPSS 8.0 statistical software package. One-way ANOVAs (Sokal and Rohlf, 1969) were done along with descriptive statistics, regression analysis and Tukey's post hoc test ($P<0.05$). Variances were tested for normality and homogeneity. Results are presented as mean ± standard error (SE).

3. Results

Fish at 28 dph from protocol G12, (253.4 ± 10.3 mg WW and 21.5 ± 0.3 mm SL), were significantly larger than those from the protocols that received *Artemia*. Protocols (G12A and P12A) that received *Artemia* and either microdiet resulted in similar growth (218.2 ± 13.7 and 209.0 ± 12.0 mg WW, respectively, and 20.2 ± 0.4 and 19.8 ± 0.4 mm SL, respectively) (Table 1 and Fig. 2).

Fish grown until 36 dph following rearing protocols G12 and G12A, showed no significant difference in size (0.71 ± 0.20 and 0.75 ± 0.20 g WW, respectively, and 36.6 ± 4.1 and 37.7 ± 3.5 mm SL, respectively).

A positive relationship (*correlation coefficient* = 0.97) between the number of days that rotifers were fed to each protocol (Fig. 1) and the final weight of the larvae at 28 dph (Table 1) was shown. The size of larvae at 10 dph

Table 1
Final wet weight of larvae at 28 dph for each protocol (mean±SE, $n=4$)

Protocol	Wet weight (mg) at 28 dph	Experimental protocol % survival	Air exposure stress % survival
G	1.3 ± 0.0^{e}	0.1 ± 0.0^{d}	0.0 ± 0.0^{d}
G3	21.3 ± 1.8^{d}	2.0 ± 0.7^{c}	2.9 ± 2.9^{c}
G7	95.4 ± 6.6^{c}	2.5 ± 1.0^{bc}	33.3 ± 12.2^{b}
G12	253.4 ± 10.3^{a}	5.9 ± 0.9^{b}	63.0 ± 4.5^{a}
G12A	218.2 ± 13.7^{b}	13.5 ± 1.3^{a}	80.8 ± 8.5^{a}
P12A	209.0 ± 12.0^{b}	8.6 ± 3.2^{ab}	72.4 ± 11.3^{a}

Protocol survival and survival post air exposure stress for protocols. Means sharing a common superscript are not significantly different ($P<0.05$).

was adversely affected by introducing the microdiet earlier. Larvae from protocol G12, in which co-feeding with rotifers and microdiet commenced at 9 dph was significantly ($P<0.05$) larger at 10 dph than larvae reared according to all other protocols that received microdiet earlier (Table 1).

Larvae in protocols (P12A and G12A) that received *Artemia* grew significantly ($P<0.05$) better than larvae in the protocol (G12) that did not receive *Artemia*, only during the period when *Artemia* were fed, as indicated by the DGC values (Fig. 3). Following the cessation of *Artemia* inputs from 22 to 28 dph, G12, G7 and G3 larvae all showed higher growth rates than P12A and G12A larvae (Fig. 3). Larvae from protocols G7, G12, G12A and P12A experienced reduced growth rates by 28.1±19.1%, 55.0±16.4%, 40.2±4.0% and 44.0±9.0%, respectively, during the period directly after live feed was terminated. The recovery in growth rate following the lag period post wean, 18–22 and 28–36 dph, respectively, for protocols G12 and G12A, was 381.7±171.1% and 214.2±36.3%, respectively (Fig. 3).

Protocol G12A larvae showed significantly ($P<0.05$) better survival than larvae in all other protocols except P12A. P12A and G12 larvae were not significantly different from each other, however P12A showed significantly ($P<0.05$) better survival than protocols G, G3 and G7 (Table 1). Protocol G12 was not significantly different from protocol G7, however it showed significantly ($P<0.05$) better survival than protocols G and G3. Protocols G7 and G3 were not significantly different, but both protocols showed significantly ($P<0.05$) better survival than protocol G, which had very few survivors to 28 dph. A regression analysis ($r^2=0.94$) showed a positive relationship between larvae survival in protocols G, G3, G7 and G12 and the exposure time to rotifers (Table 1).

G12A larvae showed the strongest resilience to stress but not significantly better than P12A and G12 larvae. The remaining protocols had significantly ($P<0.05$) less larvae survival following stress (Table 1). The larvae's resilience to stress showed a positive correlation ($P<0.05$) to the length of time that they received rotifers and/or *Artemia* and positively correlated with growth and survival data (Fig. 1).

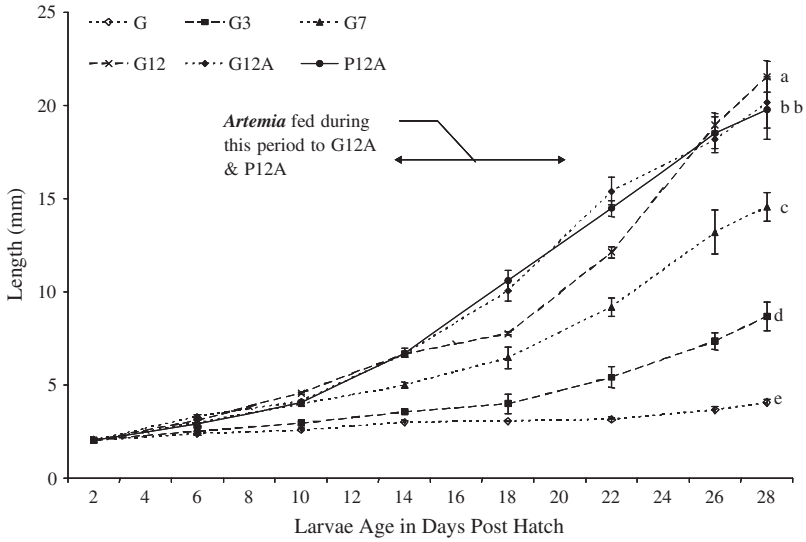

Fig. 2. Growth curve (mean standard length (SL) mm±SE, initial $n=100$, final $n=20$, intermediate $n=5$) of larvae weaned according to six different protocols, from 2 days post hatch (dph) to 28 dph. Means sharing a common superscript are not significantly different ($P<0.05$) at 28 dph. Protocol-G received only Gemma Micro (Skretting) microdiet from initial exogenous feeding, and protocols G3, G7 and G12 received rotifers for 3, 7 and 12 days, respectively, including a 3 day co-feeding period with Gemma Micro microdiet. Protocols G12A and P12A received rotifers for 12 days and *Artemia* for a further 9 days until 20 dph and either Gemma Micro microdiet or Proton (INVE) microdiet, respectively.

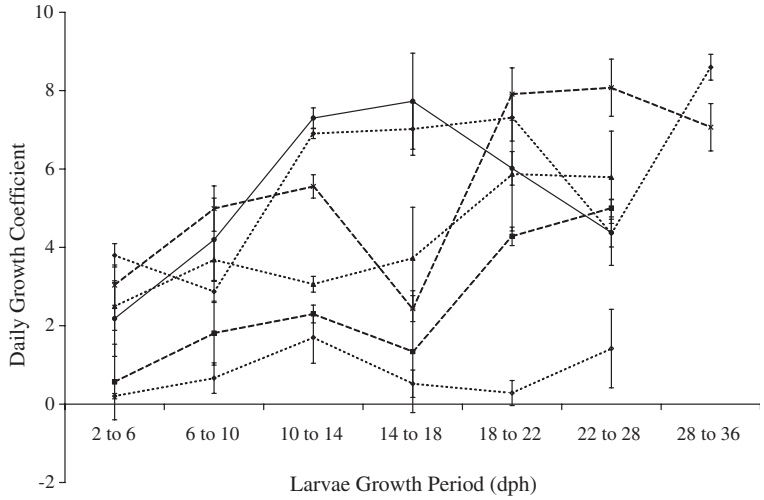

Fig. 3. Daily growth coefficient for periods between observation days. Protocols are denoted as follows, G (– –◇– –), G3 (– –■ –), G7 (–––▲–––), G12 (– –x –), G12A (–––◆–––) and P12A (—●—).

The amino acid analysis of the microdiet leaching supernatant showed that Gemma Micro leached an average of 170% the amount of amino acids into the water column than Proton. Qualitative analysis showed that Gemma Micro leached twice the number of amino acids than Proton. In particular, amino acids that are considered attractants Kolkovski (2000), such as glutamic acid, aspartic acid, threonine, alanine were found exclusively in Gemma Micro and valine, leucine, isoleucine and histidine leached at 200%, 200%, 250% and 400%, the rate respectively, compared to Proton, while taurine leached from both diets in equal quantities (Table 2).

The proximate analysis of the diets showed that the protein levels are not different. However, total lipid levels were significantly ($P<0.05$) higher in Gemma

Table 2
Concentration of individual free amino acids (μmol g^{-1}) leached from microdiets in 8 min into marine water at 21 °C

Amino acid\microdiet	Gemma 150	Gemma 300	Gemma 0.3	Proton 2	Proton 3	Proton 4
Arginine*						
Lysine*	0.6	0.6	0.5	0.6	0.6	0.5
Glutamic acid*	4.6	3.4	2.7			0.5
Aspartic acid*	1.2					
Glutamine	0.6		0.5			
Asparagine	0.6	1.1				
Histidine*	2.3		1.1	0.6	0.6	
Tyrosine	1.2	4.0				
Tryptophan	4.6	5.1	3.8	4.6	3.3	3.8
Serine						
Threonine*	1.7					
Glycine*						
Alanine*	0.6					
Methionine	10.4	1.1	1.1	1.2		1.1
Phenylalanine*						
Leucine*	19.1	15.3	7.1	9.8	7.8	4.4
Isoleucine*	12.7	11.3	3.3	4.6	4.5	2.7
Taurine*	28.9	34.5	32.3	35.2	26.7	26.8
Valine*	22.5	21.5	11.0	10.4	11.1	9.3
Citrulline						
Total	111.5	97.8	63.5	66.9	54.6	49.3

Amino acids that are known to have attractant properties are indicated with an asterisk (*) (Kolkovski, 2000, 2004).

Table 3
Proximate analysis of microdiets, (ar = as received, DW = dry weight)

Diet\parameter	Moisture	Protein (crude)	Fat
	ar (%)	DW (%)	DW (%)
Gemma Micro	6.2	58.8	19.9
Skretting crumble salmon starter	7.3	47.4	17.6
Proton	7.2	56.9	12.9

Micro than Proton at 19.9% dry weight (DW) and 12.9% DW, respectively (Table 3).

4. Discussion

4.1. Microdiet

The objective of producing hatchery-reared fish is to reliably supply high quality juveniles for growing out while minimizing the costs. The elimination of *Artemia* from commercial rearing protocols by substituting an appropriate microdiet would be considered a substantial saving on overall costs (Person-Le Ruyet et al., 1993), however larvae quality must be maintained. We have shown that barramundi larvae development is affected by rearing protocols, with larval physiology restricting live feed reduction (Walford and Lam, 1993; Cahu and Zambonino Infante, 2001).

However, co-feeding rotifers and Gemma Micro allowed complete replacement of *Artemia*, with better growth and similar survival compared to a commonly used protocol co-feeding rotifers, *Artemia* and Proton. Moreover, by co-feeding *Artemia* with Gemma Micro, survival was significantly improved. We hypothesized that superior digestibility and a heightened attraction to Gemma Micro particles in pre-metamorphic larvae was responsible for this outcome.

Chemical analysis of the diets showed that Gemma Micro leached significantly higher amounts of amino acids into the water column, and at least 7 amino acids that were not found in Proton, 4 of which are known attractants (Kolkovski, 2000). This would subsequently stimulate increased ingestion of Gemma Micro (Partridge and Southgate, 1999; Kolkovski, 2001) and improve its digestibility and assimilation (Cahu and Zambonino Infante, 2001).

Moreover, although both microdiets have similar total protein levels, Gemma Micro has significantly higher lipid levels. While, optimal total lipid levels are species specific (Brinkmeyer and Holt, 1995; Zambonino Infante and Cahu, 1999), barramundi larvae showed significantly better survival in those protocols that received higher lipid levels, with former results being similar for other species (Kanazawa et al., 1982; Kanazawa, 1993; Salhi et al., 1994; Coutteau et al., 1997).

The resulting low survival of all protocols in this trial was attributed to the late application of their first feeding. Similar results were found in commercial runs at Darwin Aquaculture Centre (NT, Australia), when the initial feeding was delayed to 2.5 dph compared to 1.5 dph, as in this trial (Bosmans et al., 2005).

4.2. Premature co-feeding

We showed that the premature introduction of microdiet, even when co-feeding with rotifers, inhibited larval growth. In addition feeding larvae exclusively microdiet prevented the onset of the maturation process allowing few survivors to 28 dph, despite being observed to ingest the microdiet. One possible explanation for this is the inability of larvae to efficiently digest the microdiet before the stomach begins to differentiate (at 5 mm) (Walford and Lam, 1993; Kolkovski, 2001). Furthermore, ingestion of an inadequate microdiet may delay or even prevent the genetically programmed sequence of intestinal maturation (Cahu and Zambonino Infante, 2001). While live feed organisms stimulate the development of the digestive system and assist in digestion by introducing exogenous digestive neuropeptides/enzymes to the larvae gut (Hjelmeland et al., 1988; Kolkovski et al., 1997b; Baskerville-Bridges and Kling, 2000; Kolkovski, 2001).

4.3. Live feed

According to the results of this trial the minimum temporal requirement for live feed inputs is 12 days, without compromising growth. However, we found that the cessation of rotifer inputs at 13 dph reduces survival, compared to completing weaning at 20 dph. Alternatively, gilthead seabream *Sparus aurata* larvae were reported to become increasingly difficult to wean the longer co-feeding is continued (Kolkovski et al., 1995; Canavate and Fernandez-Diaz, 1999). However, these results report a phenomena relating to post-metamorphic larvae. Clearly, there are two weaning effects taking place. Firstly, stimuli such as live prey have a greater influence on the feeding response of larvae in early development and the cessation of live feed would limit ingestion activity of microdiet particles (Kolkovski et al., 1997a). The improved feeding response to *Artemia* shown by Kolkovski et al. (1997a,b) relates to both visual and chemical stimuli, such as movement and the excretion of chemical attractants (betaine, free amino acids and peptides). Furthermore, an increased synergistic effect

of the stimuli was hypothesized (Kolkovski et al., 1997a, b). Therefore, although Gemma Micro was found to leach free amino acids the attractant effect would be less significant in terms of ingestion rates than the combined effect of microdiet leachate, *Artemia* movement and their associated metabolites.

Secondly, at 13 dph (5 mm) the larval gut physiology would restrict the bioavailability of nutrients bound by the microdiet particles (Walford and Lam, 1993; Cahu and Zambonino Infante, 2001). Stomach formation begins when larvae reach 5 mm but is not complete until the larvae are 12 mm (18 dph), after which gastric fluids become more acidic (Walford and Lam, 1993). This facilitates a more efficient peptic digestion and consequently increases the assimilation of microdiet nutrients (Walford and Lam, 1993; Moyano et al., 1996). Thus, the inclusion of *Artemia* in the rearing protocol significantly improved pre-metamorphic growth and survival by increasing ingestion and possibly aiding digestion by providing exogenous enzymes (Hjelmeland et al., 1988; Kolkovski, 2001). Despite this, reduced growth subsequent to weaning post metamorphosis is entirely attributed to a preference for live feed as reported in sea bream larvae (Kolkovski et al., 1997b) and subsequently a reduction in nutrient intake during a period of behavioural modification, rather than an inability to efficiently digest the microdiet (Canavate and Fernandez-Diaz, 1999; Cahu and Zambonino Infante, 2001; Kolkovski, 2001).

4.4. Compensatory growth

Regardless of the timing (dph) of weaning, larvae receiving Gemma Micro underwent a period of slow growth during weaning and then a period of accelerated or compensatory growth once the larvae were fully weaned and able to digest and assimilate microdiet nutrients. Compensatory growth is attributable to the restoration of improved nutritional intake following a period of relative starvation caused by removing the preferred live food source and then replacing it with one that is more nutritious (Tian and Qin, 2004). The scale of compensatory growth is reflected by the extent of nutritional deficiencies in the preceding period (Tian and Qin, 2004). Therefore in this experiment, the level of microdiet nutrient bioavailability post wean was lower for larvae weaned earlier. Although, relatively higher compensatory growth post metamorphosis for larvae fed rotifers for 12 days and no *Artemia* made them larger at 28 dph, the same growth effect occurred in larvae that did receive *Artemia*, which resulted in larvae of similar size at 36 dph. These results contradict those previously reported for many marine fish species using a variety of microdiets, stating that *Artemia* are essential for rearing marine fish (Izquierdo and Fernandez-Palacios, 2001; Kolkovski, 2001; Koven et al., 2001). However, given an efficient microdiet that is well adapted to larvae requirements marine fish have the potential to survive with minimal live feed inputs (Cahu et al., 1998; Cahu and Zambonino Infante, 2001).

5. Conclusion

The ideal time to complete weaning onto dry microdiet is at the earliest stage when the larvae can efficiently digest the microdiet and when live feed inputs no longer provide growth and/or survival benefits to the larvae (Kolkovski et al., 1995; Canavate and Fernandez-Diaz, 1999; Cahu and Zambonino Infante, 2001). This is based on larval organogenesis and the time of metamorphosis, after which the fish are considered to be juveniles and better able to assimilate microdiet nutrients (Walford and Lam, 1993; Cahu and Zambonino Infante, 2001). Therefore we suggest that enriched rotifers be fed at mouth opening and continued until 3 days after the initiation of stomach differentiation (at 5 mm SL). In order to stimulate feeding and reduce mortality during weaning supplemental levels of $n-3$ HUFA enriched *Artemia* should be provided (5% of previously accepted industry standard rates — 1 kg cysts per million weaned larvae (Bosmans et al., 2005), starting at 5 until 12 mm, when the stomach is fully developed. Co-feeding barramundi larvae on microdiet (i.e. Gemma Micro) should be started no earlier than 3 days prior to stomach differentiation and be continued post metamorphosis. This method improves growth by 25–30% over the previous standard method, and mortality during weaning is reduced from 5% to 1% (Bosmans et al., 2005). Furthermore, post wean growth is tremendously improved, because the larvae adapt more quickly to the nutritionally superior microdiet, rather than being satiated with protein poor *Artemia*.

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